DNA damage and repair

Introduction

DNA is the repository of genetic information in each living cell, its integrity and stability are essential to life. DNA, however, is not inert; rather, it is a chemical entity subject to assault from the environment, and any resulting damage, if not repaired, will lead to mutation and possibly disease.

DNA damage exists in all cellular organisms . While DNA damage is distinguished from mutation, mutation can result from unrepaired DNA. While most DNA damage can be repaired, such repair systems are not 100% efficient. Un-repaired DNA damage accumulates in non-replicating cells, such as neurons or myocytes of adult mammals, and can cause aging. DNA damage can be subdivided into two types:

1) endogenous damage caused by reactive oxygen species (ROS) that are derived from metabolic by product. Also includes replication error.

2) exogenous damage caused by radiation (UV, X-ray, gamma), hydrolysis, plant toxins, and viruses.

Agents that Damage DNA

1- **Highly reactive oxygen radicals produced** during normal cellular respiration as well as by other biochemical pathways

2- **Ionizing radiation** such as gamma rays and x-rays

3-**Ultraviolet rays**, especially the UV-C rays (~260 nm) that are absorbed strongly by DNA but also the longer-wavelength UV-B that penetrates the ozone shield.

٤- **Aromatic hydrocarbons**, including some found in cigarette smoke

٥-**Plant and microbial products**, e.g. the Aflatoxin

٦- **Chemicals used in chemotherapy**, especially chemotherapy of cancers.

Types of DNA damage

Type of Damage	Examples
Single-base alteration	Depurination
	Insertion or deletion of nucleotide
	Alkylation of base
Two-base alterations	UV light–induced thymine-thymine (pyrimidine) dimer
Chain breaks	Ionizing radiation
	Oxidative free radical formation
Cross-linkage	Between bases in same or opposite strands
	Between DNA and protein molecules (eg, histones)

DNA Repair

DNA repair can be grouped into two major functional categories:

A) Direct Damage reversal

B) Excision of DNA damage

A) Direct Damage Reversal

Most cases of DNA damage are not reversible. For cases that are reversible, our body uses direct reversal repair mechanism to correct the damaged base.

Direct reversal repair is a mechanism of repair where the damaged area or lesion is repaired directly by specialised proteins in our body. It is the simplest form of DNA repair and also, the most energy efficient method. It does not require a reference template unlike the other single-strand repair mechanism. Moreover, it does not involve the process of breaking the phosphodiester backbone of the DNA.

The direct reversal of the damage, which may be a more efficient way of dealing with specific types of DNA damage that occur frequently. Only a few types of DNA damage are repaired in this way, particularly pyrimidine dimers resulting from exposure to ultraviolet (UV) light and alkylated guanine residues that have been modified by the addition of methyl or ethyl groups at the O6 position of the purine ring.

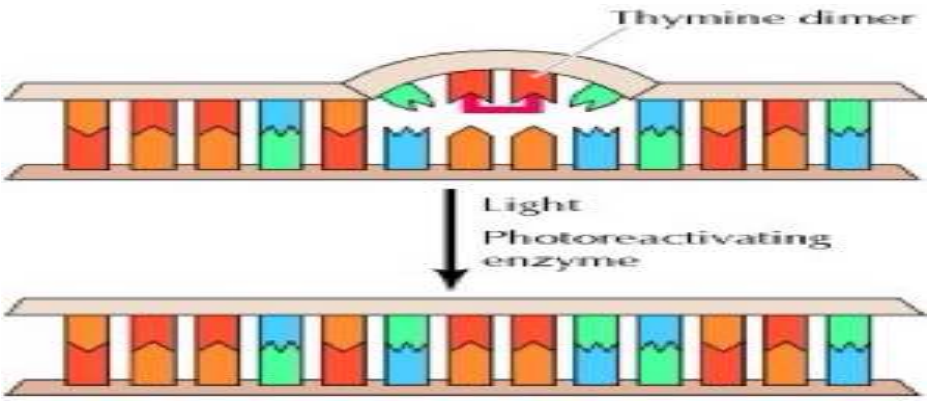

Figure 1: Direct repair of thymine dimers

An example of reversible DNA damage repairable via Direct Repair is Alkylation which can be repaired via direct removal of the Alkyl groups. Alkylating agents are carcinogens that is capable of alkylating DNA in our body. It is widely used to create medicines (e.g., treatment of leukaemia, tumors) and industrial chemicals. Alkylated DNA bases resulted in improper base pairing and ultimately, lead to cell death.

An example of Alkylation is Methylation which is the addition of a methyl group (CH3) to a guanine (G) nucleotide. This resulted in a complementary pairing to thymine (T) instead of cytosine (C).

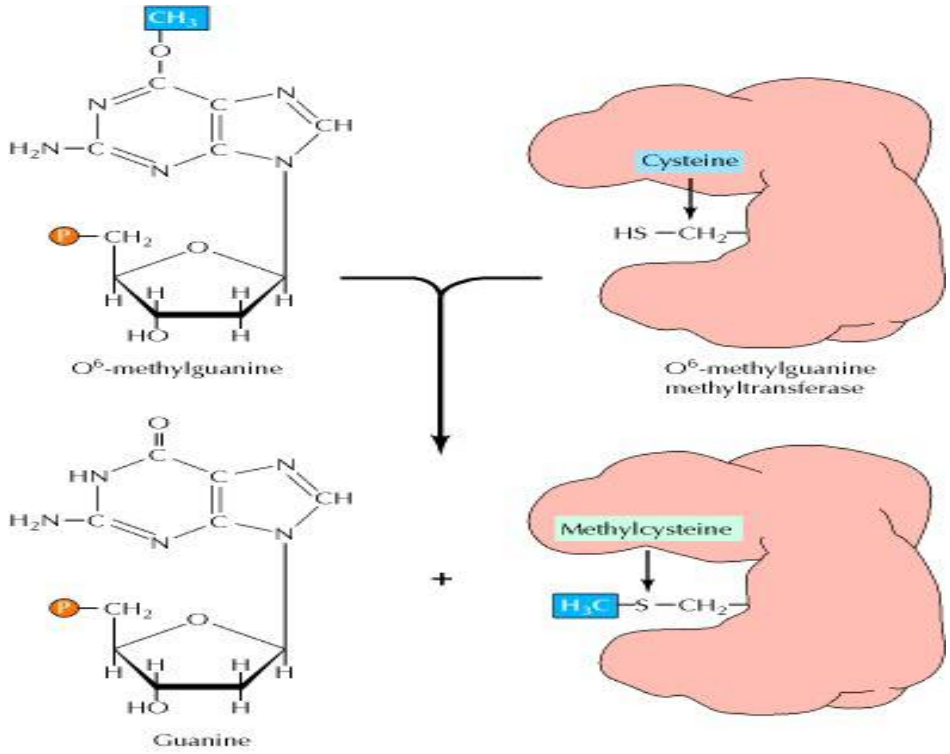

Figure 2: Repair of O6-methylguanine

O6-methylguanine methyltransferase transfers the methyl group from O6-methylguanine to a cysteine residue in the enzyme's active site

B) Excision of DNA damage

Although direct repair is an efficient way of dealing with particular types of DNA damage, excision repair is a more general means of repairing a wide variety of chemical alterations to DNA. Consequently, the various types of excision repair are the most important DNA repair mechanisms in both prokaryotic and eukaryotic cells In excision repair, the damaged DNA is recognized and removed, either as free bases or as nucleotides. The resulting gap is then filled in by synthesis of a new DNA strand, using the undamaged complementary strand as a template. Three types of excision repair—**base-excision repair , nucleotide excision repair**, and **mismatch repair** cells to cope with a variety of different kinds of DNA damage.

Base-excision repair

The repair of uracil is a good example of base-excision repair , in which single damaged bases are recognized and removed from the DNA molecule .Uracil can arise in DNA by two mechanisms: (1) Uracil (as dUTP [deoxyuridine triphosphate]) is occasionally incorporated in place of thymine during DNA synthesis, and (2) uracil can be formed in DNA by the deamination of cytosine.

The second mechanism is of much greater biological significance because it alters the normal pattern of complementary base pairing and thus represents a mutagenic event. The excision of uracil in DNA is catalyzed by DNA glycosylase an enzyme that cleaves the bond linking the base (uracil) to the deoxyribose of the DNA backbone.

This reaction yields free uracil and an apyrimidinic site—a sugar with no base attached. DNA glycosylases also recognize and remove other abnormal bases, including hypoxanthine formed by the deamination of adenine pyrimidine dimers, alkylated purines other than O6-alkylguanine, and bases damaged by oxidation or ionizing radiation.

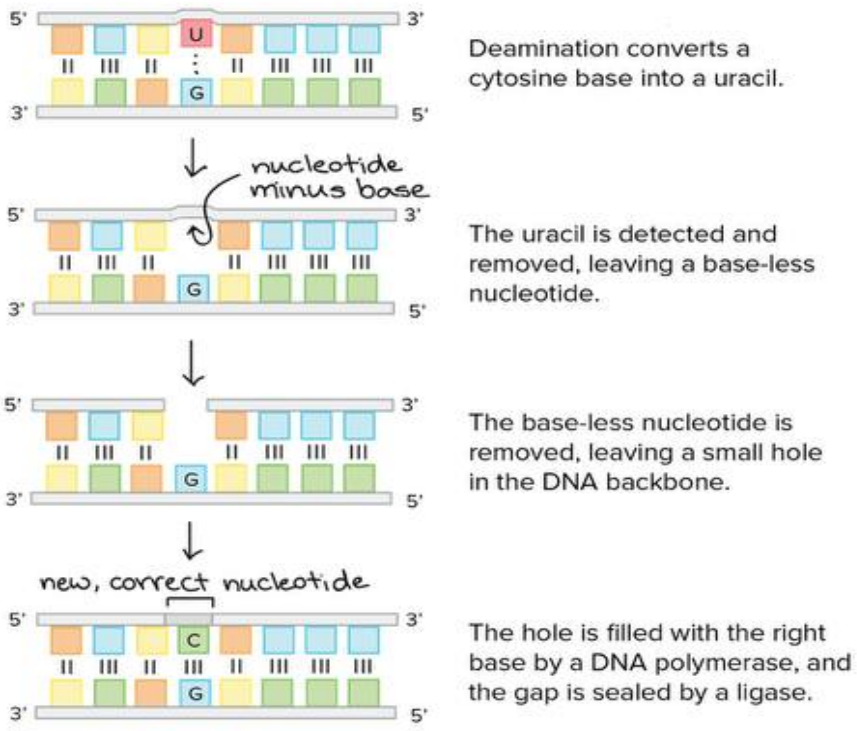

Figure 3: Base-excision repair

nucleotide excision repair

Whereas DNA glycosylases recognize only specific forms of damaged bases, other excision repair systems recognize a wide variety of damaged bases that distort the DNA molecule, including UV-induced pyrimidine dimers and bulky groups added to DNA bases as a result of the reaction of many carcinogens with DNA. This widespread form of DNA repair is known as nucleotide -excision repair, because the damaged bases (e.g., a thymine dimer) are removed as part of an oligonucleotide containing the lesion .

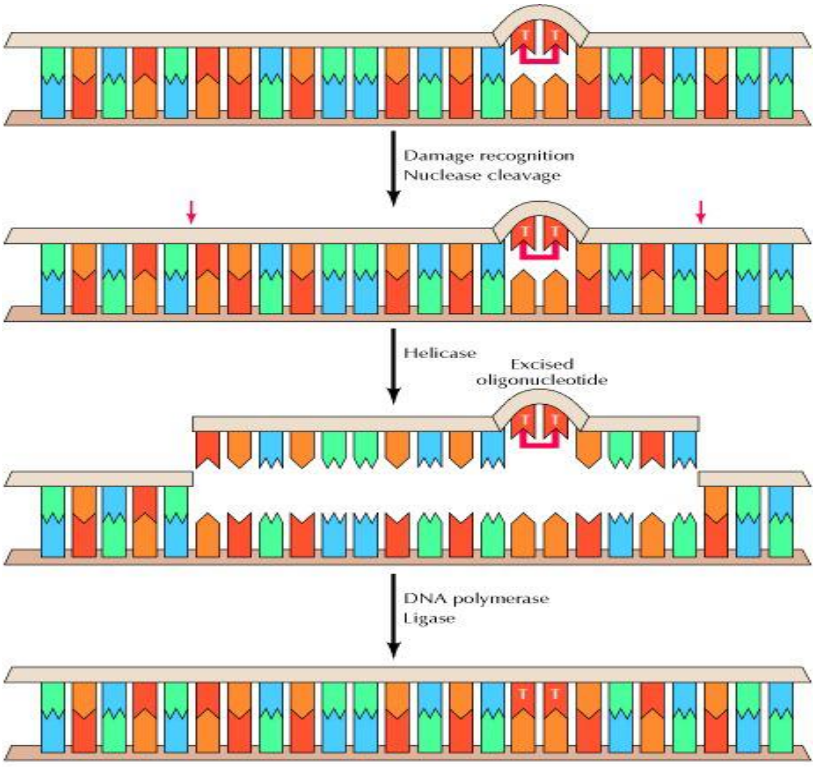

Figure 4: Nucleotide-excision repair of thymine dimers

mismatch repair

A third excision repair system recognizes mismatched bases that are incorporated during DNA replication. Many such mismatched bases are removed by the proofreading activity of DNA polymerase .The ones that are missed are subject to later correction by the mismatch repair system, which scans newly replicated DNA. If a mismatch is found, the enzymes of this repair system are able to identify and excise the mismatched base specifically from the newly replicated DNA strand, allowing the error to be corrected and the original sequence restored.

In E. coli, the ability of the mismatch repair system to distinguish between parental DNA and newly synthesized DNA is based on the fact that DNA of this bacterium is modified by the methylation of adenine residues within the sequence GATC to form 6-methyladenine . Since methylation occurs after replication, newly synthesized DNA strands are not methylated and thus can be specifically recognized by the mismatch repair enzymes. Mismatch repair is initiated by the

protein MutS, which recognizes the mismatch and forms a complex with two other proteins called MutL and MutH. The MutH endonuclease then cleaves the un methylated DNA strand at a GATC sequence. MutL and MutS then act together with an exonuclease and a helicase to excise the DNA between the strand break and the mismatch, with the resulting gap being filled by DNA polymerase and ligase.

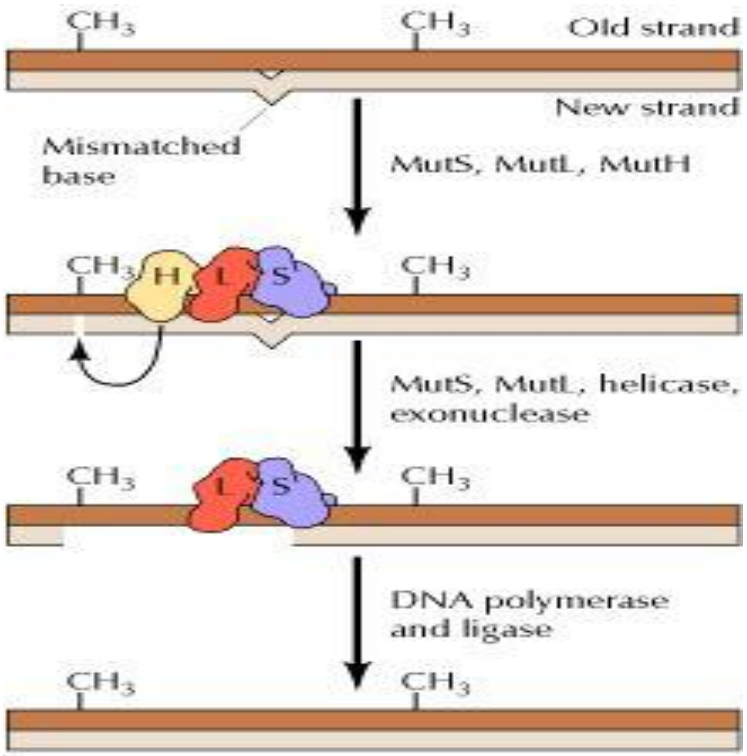

Figure 5: Mismatch repair in E.coli

Mismatch repair in mammalian cells is similar to E. coli, except that the newly replicated strand is distinguished from the parental strand because it contains strand breaks. MutS and MutL bind to the mismatched base and direct excision of the DNA between the strand break and the mismatch.

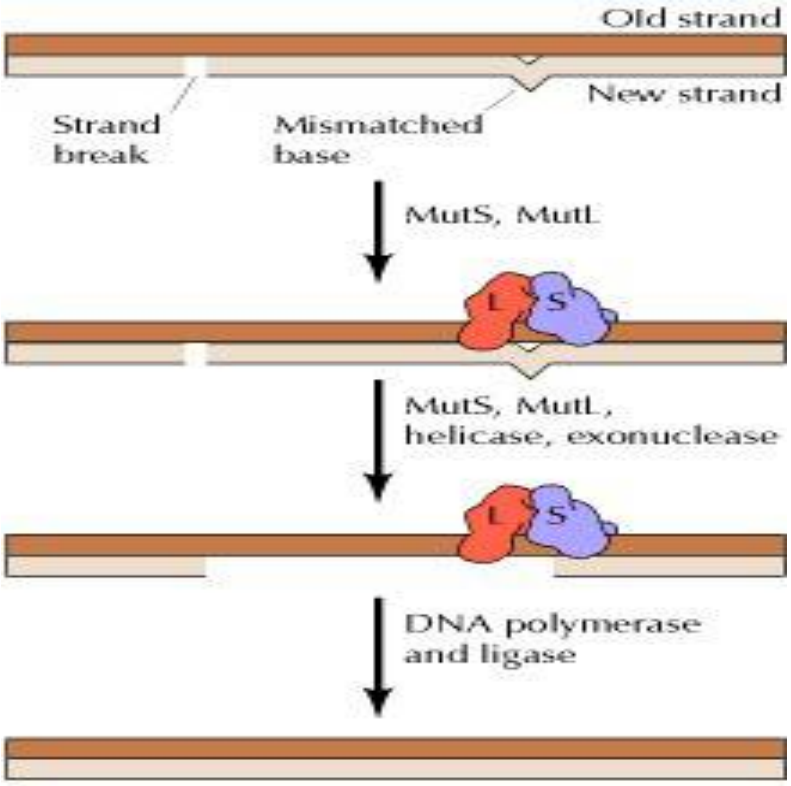

Figure 5: Mismatch repair in mammalian cells

Lecture 8 : DNA damage and repair

Presented by: Safaa Abbass Abd Al- kahdum